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NEW PEPTIDES AND USE THEREOF

Field of the invention

The present invention relates to new peptides and to use thereof, in particular for treatment and/or prevention of infections, inflammations and/or tumours.

Background art

It has for a long time been known that human milk in several ways is anti-inflammatory due to the fact that it is poor in initiators and mediators of inflammation but rich in anti-inflammatory agents (see e.g. Goldman A. S., et al., Anti-inflammatory properties of human milk, Acta Paediatr. Scand. 75:689-695, 1986). Human milk also contains several soluble anti-infective components, such as lactoferrin (see e.g. Hanson L. Å., et al., Protective factors in milk and the development of the immune system, Pediatrics 75:172-176, 1983).

Lactoferrin is a single chain metalbinding glycoprotein with a molecular weight of 77 kd. It has been found that the structural domain of lactoferrin responsible for the bactericidal properties is a pepsin-cleaved fragment called lactoferricin (see e.g. Bellamy W., et al., Identification of the bactericidal domain of lactoferrin, Biochim. Biophys. Acta 1121:130-136, 1992, and Bellamy W., et al., Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin, J. Appl. Bact. 73:472-479, 1992).

Lactoferrin receptors are found on many types of cells including monocytes and macrophages, lectin-stimulated human peripheral blood lymphocytes, brush-border cells, and tumour cell lines.

Several patent publications describe the possible use of lactoferrin for treatment of infections or inflammations. In WO 98/06425, e.g., it is disclosed that lac-

toferrin and lactoferricin can be used for treatment and prevention of infections, inflammations and tumours.

EP-A-0 629 347 describes an antimicrobial agent containing (A) lactoferrin hydrolysate and/or one or more of antimicrobial peptides derived from lactoferrins, and (B) one or more compounds selected from the group consisting of metal-chelating protein, tocopherol, cyclodextrin, glycerin-fatty acid ester, alcohol, EDTA or a salt thereof, ascorbic acid or a salt thereof, citric acid or a salt thereof, polyphosphoric acid or a salt thereof, chitosan, cysteine, and cholic acid as the effective components thereof. This antimicrobial agent is intended for treatment of products, and especially for safely treating e.g. food and medicines. The agent according to this publication is thus a new preservative. In the publication several peptide sequences are given and some of them resemble the peptides according to the invention, although there are several important differences described further below. The peptides described in this publication also have different functions than the peptides according to the invention, which are shown below in the comparative examples.

Even though native human lactoferrin and lactoferricin have been shown to have desired anti-inflammatory, anti-infectious and anti-tumoural properties they cannot be used clinically on a broad basis since they are very expensive substances due to high production costs.

Summary of the invention

The object of the present invention is to provide new peptides which can be used for the same purposes as lactoferrin and/or lactoferricin and which will have the same, or better, effects although being much cheaper to produce.

The aim of the studies leading to the present invention was to design new peptides which could be taken up from the intestines. It has been shown that humans in

their brush border membrane have receptors which can bind to human lactoferrin (see e.g. Lönnerdal B., Lactoferrin receptors in intestinal brush border membranes, Adv. Exp. Med. Biol., 1994, 357:171-175). It has also been shown that bovine lactoferrin does not bind to these receptors. The new peptides should therefore resemble human lactoferrin or human lactoferricin but they should also be easier and especially cheaper to produce. Furthermore, they should be essentially as efficient as, or preferably more efficient than human lactoferrin or human lactoferricin in treatment and prevention of infections, inflammations and tumours.

It was found that the peptides formed of the sequences constituted of amino acids 16-40 and amino acids 18-40 from the N-terminal end of human lactoferrin, with some alterations described further below, have the desired properties. Also sequences with only 14 residues, roughly corresponding to residues 18-31 of human lactoferrin wherein C-20 is replaced by A, Q-22 is replaced by K, and N-26 is replaced by D, were found to have the same, and even better, properties.

A plausible mechanism for the uptake of these new peptides in the human body is that the peptides are taken up in the intestine through binding to the above mentioned specific lactoferrin receptors and are then transported through the circulation. However, the invention is in no way limited to this mechanism.

Thus, the present invention relates to new peptides with the sequences given in the appended sequence listing, and to functionally equivalent homologues or analogues thereof.

Furthermore, the invention relates to medicinal products and to food stuff, especially infant formula food, comprising said peptides.

The invention also relates to use of said peptides for the production of medicinal products for treatment and prevention of infections, inflammations and tumours.

The characterising features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

Thus, the invention relates to peptides the sequence of which is:

Ac-X_1-X_2-T-K-X_3-F-X_4-W-Q-R-X_5-M-R-K-V-R-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-NH_2

(sequence No. 1)

wherein X_1 is either E or no amino acid, X_2 is either A or no amino acid, X_3 is either C or A, X_4 is either Q or K, X_5 is either N or D, and X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14} is either -G-P-P-V-S-C-I-K-R or no amino acid. In all sequences herein single-letter symbols are used to denote the amino acids. These symbols, which are known to man skilled in the art, have the following meaning: A = alanine, C = cysteine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, I = isoleucine, K = lysine, M = methionine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan. Ac and NH_2 denotes an acetyl (CH_3CO-) group and an amino group, respectively, that have been used to modify the amino and the carboxy terminals of the peptides into formation of amides, as described below.

The peptides according to the invention is either of a linear or a circular form. The linear form of the sequences No. 2 and 4 is obtained through protection of the cysteine side chains by acetamidomethyl groups $CH_3CONHCH_2$-. The circular form of the sequences No. 3 and 5 is obtained by creation of a disulphide bridge between the two cysteines. This has to be performed in a controlled way in order to avoid formation of polymers. The peptide with sequence No. 7 is obtained through formation

of a lactam between the sides chains of one of the lysines and the aspartic acid; this is further explained in example 2 below.

In one preferred embodiment of the invention X_1 is E, X_2 is A, X_3 is C, X_4 is Q, X_5 is N, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is -G-P-P-V-S-C-I-K-R, which gives a peptide the sequence of which is:

Ac-E-A-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I-K-R-NH_2
(sequence No. 2)

Another preferred embodiment of the invention is a cyclic form of this sequence obtained by the creation of a disulphide bridge, resulting in the cyclic peptide with sequence 3. The peptide is illustrated schematically below and in the appended sequence listing:

```
                 F-Q-W-Q-R-N-M-R-K-V-R-G P-P-V-S
                 |                             |
Ac-E-A-T-K-C^S---------------------------------^SC-I-K-R-NH2
```
(sequence No. 3)

Another preferred embodiment of the peptide according to the invention is a somewhat shorter peptide wherein X_1 is none, X_2 is none, X_3 is C, X_4 is Q, X_5 is N, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is -G-P-P-V-S-C-I-K-R:

Ac-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I-K-R-NH_2
(sequence No. 4)

Yet another preferred embodiment of the invention is a cyclic form of this sequence obtained by the creation of a disulphide bridge:

```
          F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S
          |                             |
Ac-T-K-C^S-----------------------------^SC-I-K-R-NH2
```

(sequence No. 5)

An even more preferred embodiment of the invention is shorter peptide in which X_1 is none, X_2 is none, X_3 is A, X_4 is K, X_5 D, and X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14} is none:

Ac-T-K-A-F-K-W-Q-R-D-M-R-K-V-R-NH_2

(sequence No. 6)

This peptide also occurs in a form wherein the residues K in position 5 and D in position 9 have been linked by the formation of a lactam between the side chains of the residues:

```
               W-Q-R
               |   |
Ac-T-K-A-F-K---D-M-R-K-V-R-NH2,
```

(sequence No. 7)

The lactam formation in this peptide between amino acid chains that are four residues apart in the sequence forces the peptide to adopt a three-dimensional structure that resembles that of the fragment 18-31 of human lactoferrin and is designed to bind better to the receptor. This last peptide with sequence No. 7 is the most preferred embodiment of the invention.

One advantage of the peptides with sequences No. 6 and 7 compared to the other peptides according to the invention is that they are easier to synthesise and also cheaper per gram since they are shorter.

In all seven peptides the amino and carboxy terminal ends have been capped, i.e. the free NH_2 group at the amino terminal end have been reacted with acetylimidazole to form the amide CH_3CONH- or AcNH- and the free COOH at the carboxy terminal end has been transformed into $CONH_2$. Under physiological conditions at a pH of approximately

7, free amino and carboxy terminals would be ionised and the peptide would thus carry a positive and a negative charge. The capping of the ends of the peptides according to the invention makes them neutral and uncharged and thus drastically changes the electrostatic properties of the peptides. Assuming that the receptors bind the corresponding sequences of human lactoferrin where there are no N- and C terminal charges, the capped peptides should bind better as they in this respect resemble the native protein more than uncapped peptides. These capped ends constitute an important feature of the peptides according to the invention. The similar peptides described in EP-A-0 629 347 comprises free amino and carboxy terminals, and this may be one of the reasons that those peptides are in some cases not as efficient as the peptides according to the invention.

As evident from the sequences above all seven peptides according to the invention comprise the residues K and R at the carboxy terminal ends. These residues are positively charged under physiological conditions and are capable of strong and specific interactions with receptors. They are therefore an important part of the peptides according to the invention. Also the T residue at the amino terminal end of all of the peptides according to the invention is capable of playing an important part in receptor binding.

The sequences are also given in the appended sequence listing.

Apart from the above specified peptides it is also possible to use functionally equivalent homologues or analogues thereof, including those that mimic the three-dimensional structure of the corresponding segment in human lactoferrin due to the introduction of structural constraints such as lactam bridges or other chemical contraints.

The peptides according to the invention is suitable for treatment and/or prevention of infections, inflamma-

tions and/or tumours. The term "treatment" used herein refers to curing, reversing, attenuating, alleviating, minimising, suppressing or halting the deleterious effects of a disease state, disease progression or other abnormal condition, and the term "prevention" used herein refers to minimising, reducing or suppressing the risk of developing a disease state or progression or other abnormal or deleterious conditions.

The infections treatable with the peptides or medicinal products according to the invention include infections caused by all kinds of pathogens, such as bacteria, viruses, fungi, etc.

It is also possible to treat different types of inflammations. Inflammation is a complex phenomenon marked i.a. by abnormal "redness" and swelling of tissues and organs, pain and heat in affected areas, capillary dilation, leucocyte infiltration, etc. Inflammation is primarily caused by exposure to bacterial and other noxious agents and physical injury. Inflammation has many forms and is mediated by a variety of different cytokines and other chemical signals. These mediators of inflammation include tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and various colony-stimulating factors (CSFs).

As stated above, the peptides according to the invention are also suitable for treatment of tumours.

The peptides according to the invention may either be used as they are or be included in a medicinal product or a pharmaceutical preparation. The medicinal product or a pharmaceutical preparation according to the invention may also comprise substances used to facilitate the production of the pharmaceutical preparation or the administration of the preparations. Such substances are well known to people skilled in the art and may for example be pharmaceutically acceptable adjuvants, carriers and preservatives.

The peptides or medicinal products according to the invention can be administered to a patient either systemically or locally. The term "patient" used herein relates to any person at risk for or suffering from a disease state, disease progression or other abnormal or deleterious condition.

The systemic administration is suitable e.g. for treatment of urinary tract infection, colitis and tumours. The systemic administration can be undertaken by oral, nasal, intravenous, intraartery, intracavitary, intramuscular, subcutaneous, transdermal, suppositories (including rectal) or other routes known to those of skill in the art. Oral administration is preferred.

The local administration is suitable e.g. for treatment of skin infections, all infections and inflammations in mucosal membranes etc. The local administration can be undertaken by topical, oral, nasal, vaginal or oropharyngal route. For treatment of local infections or inflammations in the skin or mucosal membranes the peptides or medicinal products according to the invention may e.g. be included in a gel, a cream, an ointment, or a paste.

In the method according to the invention an effective amount of a peptide according to the invention is administered to a patient. The term "effective amount" used herein relates an amount sufficient to treat or prevent a disease state, disease progression or other abnormal or deleterious condition.

The peptides or medicinal products and methods according to the invention are particularly well suited for treatment and/or prevention of urinary tract infection and colitis, but several other inflammatory and infectious diseases are also treatable according to the present invention, such as inflammatory bowel diseases, rheumatoid arthritis, conditions caused by the virus HIV-1, conditions caused by the virus CMV, and conditions caused by the fungi Candida albicans and Candida krusei. This listing is in no way limiting the scope of the invention.

The peptides, medicinal products and methods according to the invention are also well suited for preventive medical care by reducing the risk of developing urinary tract infection or other inflammatory or infectious diseases in patients with an increased risk of attracting such complications.

The peptides, medicinal products and methods according to the invention may either be used alone, in combination with each other or in combination with conventional therapy.

According to the present invention it is also possible to include the peptides, in an effective amount, in any kind of food or beverage intended to reduce infections and/or inflammations in patients running an increased risk of such conditions due to an underlying disease or a medical treatment. For example, it is possible to include the peptides, in an effective amount, in an infant formula food intended to inhibit harmful effects of bacteria, such as weight loss caused by inflammation induced by bacteria, viruses or fungi in infants.

Since the peptides according to the invention have antimicrobial effects they can also be used as preservatives in different food stuffs and medicinal products such as gels, creams, ointments, pastes, solutions, emulsions etc.

The invention will now be further explained in the following examples. These examples are only intended to illustrate the invention and should in no way be considered to limit the scope of the invention.

Brief description of the drawing

In one of the examples below, reference is made to the appended drawing on which figure 1 shows the number of bacteria (CFU) present in kidney in mice with urinary tract infection treated with two peptides according to the invention, peptide 3 and peptide 4, with human lactoferrin, hLF, and with water, respectively.

Examples

In the examples below peptide 2 denotes the peptide according to the invention with sequence No. 2, peptide 3 denotes the peptide according to the invention with sequence No. 3, peptide 4 denotes the peptide according to the invention with sequence No. 4, peptide 5 denotes the peptide according to the invention with sequence No. 5, peptide 6 denotes the peptide according to the invention with sequence No. 6, and peptide 7 denotes the peptide according to the invention with sequence No. 7.

Morinaga 10, Morinaga 11, Morinaga 12, Morinaga 13, Morinaga 24 and Morinaga 25 denotes the peptides described in EP-A-0 629 347 with sequence No. 10 (F-Q-W-Q-R-N), sequence No. 11 (F-Q-W-Q-R), sequence No. 12 (Q-W-Q-R), sequence No. 13 (W-Q-R), sequence No. 24 (K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I), and the cyclic peptide with sequence No. 25 (K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I) (the sequence numbers are the numbers used in Morinagas patent publication EP-A-0 629 347).

hLF denotes human lactoferrin.

Example 1

This example illustrates solid phase synthesis of peptide 2, peptide 3, peptide 4 and peptide 5 according to the invention, and also of the peptides Morinaga 24 and Morinaga 25 used in the examples further below.

The syntheses were performed by Fmoc continuos flow strategy on a Biosearch Pioneer automated peptide synthesiser. The peptides were synthesised on a 0.1-0.2 mmol scale with the resins PAC-PEG-PS, 0.21 mmol/g for the peptide acids and Fmoc-PAL-PEG-PS, 0.20 mmol/g for the peptide amides.

The side chains of the peptides according to the invention were protected by piperidine-stable tert-butyl (for serine and threonine), tert-butyl ester (for glutamic acid), tert-butyloxycarbonyl (for lysine and tryp-

tophan), triphenylmethyl (for asparagine, cysteine, glutamine and histidine), acetamidomethyl (for cysteine), and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl (for arginine) groups.

For the peptides Morinaga 24 and Morinaga 25, attachment of isoleucine to the PAC-PEG-PS resin was performed using the isoleucine symmetrical anhydride. The resin (1 g, 0.21 mmol) was allowed to swell in 3 ml dimethyl formamide (DMF). Fmoc-I (10 eq, 2,1 mmol) was dissolved in 5 ml dichloromethane (DMC) and 5 drops of DMF. Diisopropyl carbodiimide (DIPCDI) (5 eq, 1.05 mmol) was added to the amino acid solution after which it was left to stand for 20 min at 0°C. The DCM was removed under reduced pressure and the remaining oil was dissolved in DMF and added to the resin. Dimethylaminopyridine (DMAP) (1 eq) was added to the resin and the slurry was allowed to stand at room temperature with occasional swirling for 1 hour. After washing with DCM the resin was ready for peptide synthesis.

Removal of the α-amino protecting group (Fmoc) was performed with 20% piperidine in DMF for 7 min. All couplings proceeded in DMF, using 4 times excess of activated amino acid over peptide and 4 eq of benzotriazolyl tetramethyluronium tetrafluoborate (TBTU):diisopropyl ethylamine (DIPEA) (1:2, mol/mol). A six fold excess of hydroxybenzotriazole (HOBt) was added to the couplings of the cysteine residues (acetamidomethyl and triphenylmethyl).

Peptide 2, peptide 3, peptide 4, and peptide 5 were acylated at the amino terminus using a 0.3 M solution of acetic anhydride in DMF.

Final deprotection and cleavage of the peptide from the resin was performed for 2 hours at room temperature using a mixture of 9.25 ml trifluoro acetic acid (TFA), 250 µl water, 250 µl ethanedithiol and 250 µl triisopropylsilan per g of peptide resin. The resin was removed by filtration. The peptide was precipitated by use of cold

diethylether, centrifuged and resuspended in fresh diethylether two more times to extract the scavengers and TFA. The peptides were dissolved in water and lyophilised.

The cyclisation of the disulphide bonded peptides, peptide 3 and peptide 5, were performed on unpurified material. Approximately 200 mg of peptide was dissolved in 1 l of degassed water. Ammonium hydrogen carbonate was added until the pH was in the range of 6-7 and the mixture was left with stirring and air contact for about 1 day, and was finally lyophilised.

The peptides were purified by reversed-phase high-pressure liquid chromatography eluating with isochratic mixtures of isopropanol (12-16% IPA) and 0.1% TFA. Two different columns were used, Microsorb, C-8 41.4x250 mm, 8 µm (column A), and Hichrom, C-8 25x250 mm, 7 µm (column B). The peptides eluated as broad peaks with the retention times (R) given in table 1 below. The identification was done by ES-MS. MW in the table denotes the molecular weight.

Example 2

This example illustrates solid phase synthesis of peptide 6 and peptide 7 according to the invention.

The syntheses were performed as described in example 1 with the following modifications of the synthetic procedure. The side chains of the lactam forming amino acids K in position 5 and D in position 9 were protected by 1(4,4-dimethyl-2,6 dioxocyclohex-1-ylidene)ethyl (Dde) and 4-{N-[1(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino}benzyl ester (ODmab), respectively.

After the synthesis of the capped sequence was completed, the Dde and ODmab groups were removed by 2% hydrazine (v/v) in DMF for 10 min. The resin was washed with DMF, 1M DIPEA in DMF and finally by DMF.

In the case of peptide 7 lactam formation between the side chains was allowed to proceed for 8 hours after the addition of a four fold excess of azabenzotriazolyl

tetramethyluronium hexafluorophosphate (HATU):DIEPEA, 1:2 in DMF. The resin was washed with the following solvents/solutions: DMF, 20% piperidine in DMF, methanol and DCM. The resin was dried under vacuum, and the peptide was cleaved from the resin as described above.

Peptide 7 eluated as a broad peak with the retention times (R) given in table 1 below.

Table 1

Peptide	Column	% IPA	Flow (ml/min)	R (min)	MW (found/calc.)
Peptide 2	B	14	15	11	3201/3204.7
Peptide 3	A	13	80	16	3057.1/3060.7
Peptide 4	A	14	80	12	3002.4/3004.0
Peptide 5	B	14	15	10	not determined
Peptide 7	B	12	18	12	not determined
Morinaga 24	B	16	15	20	2575/2576.0
Morinaga 25	B	16	15	18	3430/3432.0

Example 3

In this example the bactericidal activity of the peptides according to the invention was tested and compared to the bactericidal activity of human lactoferrin.

Human lactoferrin (hLF), peptide 3, and peptide 4, respectively, were incubated with two different strains of E. coli, E. coli O14 (experiment I) and O6K5 (experiment II), in 1% growth medium (BHI - brain heart infusion) for 2 hours. The peptides were also incubated with E. coli O14 in 0.05 mM KCl (phosphate buffer, pH 7) without any growth medium (experiment III). Different concentrations of the peptides were tested.

After the incubation, samples were taken for bacterial plating. Serial dilutions of five- and fourfold steps were used in experiments I and II, respectively, and of twofold steps in experiment III.

The concentrations of the different peptides required for killing 100% (experiments I and II) or 90% (experiment III) of the bacteria are given in table 2.

Table 2

Exp.	Agent	Concentration of agent for 100%/90% killing (µg/ml)
I	hLF	400
I	peptide 3	4.4
I	peptide 4	4.4
II	hLF	2000
II	peptide 4	≤ 7.8
III	hLF	> 4000
III	peptide 3	12.5
III	peptide 4	3.2

From table 2 it is evident that the peptides according to the invention were much more efficient as bactericidal agents than human lactoferrin.

Example 4

In this example the fungicidal activity of the peptides according to the invention was tested and compared to the fungicidal activity of human lactoferrin. Different concentrations of hLF, peptide 3 and peptide 4 were incubated with different strains of Candida albicans and Candida krusei during 1-2 hours at 37°C in a phosphate buffer at pH 7.0 with 0.05 mM KCl at two different occasions - experiment I and II, respectively. After incubation samples were taken for plating on Saboroud plates. The concentrations of the different peptides required for killing 99% of the fungi are given in table 3.

Table 3

Exp.	Candida strain	Concentration of agent for 99% killing (µg/ml)			ratio hLF/	
		hLF	peptide 3	peptide 4	/pep. 3	/pep. 4
I	C. albicans ATCC 64549	> 50	0.6	1.25	> 83	> 40
I	C. albicans CCUG 90028	> 50	0.6	1.25	> 83	> 40
I	C. krusei CCUG A	12.5	0.6	1.25	21 .	10
I	C. krusei CCUG 969	25	1.25	2.5	20	10
II	C. albicans ATCC 64549	> 200	1.5	1.5	> 133	> 133
II	C. albicans CCUG 599	> 200	1.5	1.5	> 133	> 133
II	C. albicans CCUG 1759	> 200	1.5	1.5	> 133	> 133

The results in table 3 show that the peptides according to the invention were much more efficient fungicidal agents than human lactoferrin.

Example 5

In this example an in vitro test was performed to study the anti-inflammatory activity of the peptides according to the invention. More precisely, the inhibitory effect of the peptides according to the invention on the LPS-induced IL-6 response in a monocytic cell line (THP-1) was studied and compared to the effect of human lactoferrin. The IL-6 response in the THP-1 cells was induced by addition of LPS. hLF, peptide 3 and peptide 4, respectively, were added 30 minutes after LPS. A significant inhibition was obtained with peptide 4, as shown in

table 3 below. The inhibitory activity of peptide 3 was similar the inhibitory activity of human lactoferrin.

Table 4

Agent	% inhibition of LPS response
hLF	15
peptide 3	17
peptide 4	39

Example 6

Peptide 3 and peptide 4 according to the invention were also tested in an in vivo study to show their effect on urinary tract infection.

E. coli O6K5 was instilled into the urinary bladder of mice. 30 minutes after instillation the different agents specified in table 5 were administered orally in an amount of 500 µg per mouse and 24 hours after instillation the number of bacteria (CFU) present in bladder and in kidney was determined. The result is shown in table 5.

The control group consisted of 10 animals in experiment I and of 23 animals in experiment II. The animals in the control groups were given tap water instead of peptide or hLF.

Table 5

Exp	Agent	Mouse strain	No. of animals	Statistical comparison* of treatment group with control group of the number of bacteria, CFU, present in kidney
I	peptide 4	CH/HeN	11	p = 0.0137
II	peptide 3	C3H/Tif	23	p = 0.0574
II	peptide 4	C3H/Tif	23	p = 0.0102
II	hLF	C3H/Tif	23	p = 0.006

* Mann-Whitney

The results from experiment II are also illustrated in figure 1.

Thus, the peptides according to the invention is capable of reducing the number of the bacteria in kidney.

Example 7

In this example an in vitro test was performed to compare the bactericidal and fungicidal activity of the peptides according to the invention with peptides described in EP-A-0 629 347. The peptide according to the invention used was peptide 4, and the peptides according to EP-A-0 629 347 called Morinaga 10, Morinaga 11, Morinaga 12, and Morinaga 13.

The peptides were incubated with E. coli O14 and Candida albicans. Two concentrations of C. albicans yeast cells were tested, $5 \cdot 10^6$ and $5 \cdot 10^3$ per ml. Different concentrations of the peptides were tested.

After the incubation, samples were taken for bacterial plating. Serial dilutions of tenfold steps were used in the experiments marked with I in table 5 and of twofold steps in the experiments marked with II.

The concentrations of the different agents required for killing of 100% of the bacteria are given in table 6.

Table 6

Agent	Concentration of agent for 100% killing (µg/ml)			
	E. coli 014		C. albicans	
	I	II	I	
			$5\cdot10^6$	$5\cdot10^3$
peptide 4	> 10, <100	12	> 10, <100	10
hLF	nd	nd	> 1000	> 2000
Morinaga 10	> 500	1000	> 2000	> 2000
Morinaga 11	> 500	nd	> 2000	> 2000
Morinaga 12	> 500	nd	> 2000	> 2000
Morinaga 13	> 500	nd	> 2000	> 2000

nd = not determined

From table 6 it is evident that the peptide according to the invention is a much more efficient bactericidal agent than the short peptides described in EP-A-0 629 347 and than human lactoferrin.

Example 8

The fungicidal and inhibitory activity of the peptide 2, peptide 3, peptide 4, and peptide 7 according to the invention were compared to the peptides described in EP-A-0 629 347 most resembling the peptides according to the invention, i.e. Morinaga 24 and Morinaga 25.

Candida albicans ATCC 64549 ($1\cdot10^5$/ml) was incubated in the presence of the peptides during 2 hours at 37°C in 1% growth medium (BHI, twofold serial dilutions starting with 50 µg/ml). The fungicidal activity was measured by culturing 5 µl from each incubation well on Saboroud agar plates. The concentrations of the different agents required for killing of 100% of the bacteria is given in table 7.

The inhibition of growth was measured spectrophotometrically after incubation during 20 hours. The concentration of agent needed for inhibition of growth is given in table 7.

Table 7

Agent	**Concentration of agent for 100% killing (µg/ml)**	**Concentration of agent for inhibition of growth (µg/ml)**
Peptide 2	6.2	6.2
Peptide 3	6.2	6.2
Peptide 4	6.2	6.2
Peptide 7	3.1	1.5
Morinaga 24	12.5	12.5
Morinaga 25	6.2	6.2

This example show that peptide 2, peptide 3, and peptide 4 according to the invention is more efficient with regards to fungicidal and inhibitory activity than the linear peptide Morinaga 24 and that peptide 7 according to the invention is an even better fungicidal agent and inhibitor of growth of fungi.

Example 9

Also the bactericidal and inhibitory activity of the peptide 2, peptide 3, peptide 4, and peptide 7 according to the invention were compared to the activities of Morinaga 24 and Morinaga 25.

E. coli O14 was incubated in the presence of the peptides during 2 hours at 37°C in 1% growth medium (BHI, twofold serial dilutions starting with 100 µg/ml). The bactericidal activity was measured by culturing 5 µl from each incubation well on blood agar plates. The concentrations of the different agents required for killing of 100% of the bacteria is given in table 8.

The inhibition of growth was measured spectrophotometrically after incubation during 20 hours. The concentration of agent needed for inhibition of growth is given in table 8.

Table 8

Agent	Concentration of agent for 100% killing (μg/ml)	Concentration of agent for inhibition of growth (μg/ml)
Peptide 2	25	6.2
Peptide 3	12.5	3.1
Peptide 4	12.5	3.1
Peptide 7	12.5	≤ 1.5
Morinaga 24	25	6.2
Morinaga 25	12.5	3.1

This example show that peptide 2, peptide 3 and peptide 4 according to the invention have bactericidal and inhibitory effects that are approximately the same as those for Morinaga 24 and Morinaga 25, but that peptide 7 is much more efficient with regards to inhibition of growth of bacteria.

SEQUENCE LISTING

Sequence No. 1:

Ac-X_1-X_2-T-K-X_3-F-X_4-W-Q-R-X_5-M-R-K-V-R-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-NH_2

Sequence No. 2:

Ac-E-A-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R-G P-P-V-S-C-I-K-R-NH_2

Sequence No. 3:

```
                  F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S
                  |                              |
Ac-E-A-T-K-C^S-----------------------------------^SC-I-K-R-NH2
```

Sequence No. 4:

Ac-T-K-C-F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S-C-I-K-R-NH_2

Sequence No. 5:

```
            F-Q-W-Q-R-N-M-R-K-V-R-G-P-P-V-S
            |                              |
Ac-T-K-C^S---------------------------------^SC-I-K-R-NH2
```

Sequence No. 6:

Ac-T-K-A-F-K-W-Q-R-D-M-R-K-V-R-NH_2

Sequence No. 7:

```
                   W-Q-R
                   |   |
Ac-T-K-A-F-K———D-M-R-K-V-R-NH2
```

CLAIMS

1. A peptide the sequence of which is sequence No. 1 given in the sequence listing wherein X_1 is E or none, X_2 is A or none, X_3 is C or A, X_4 is Q or K, X_5 is N or D, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is -C-P-P-V-S-C-T-K-R or none, or functionally equivalent homologues or analogues thereof.

2. A peptide according to claim 1, wherein X_1 is E, X_2 is A, X_3 is C, X_4 is Q, X_5 is N, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is -G-P-P-V-S-C-T-K-R, the sequence thus being sequence No. 2 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

3. A peptide according to claim 2 which is cyclised through a disulphide bridge, the sequence thus being sequence No. 3 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

4. A peptide according to claim 1, wherein X_1 is none, X_2 is none, X_3 is C, X_4 is Q, X_5 is N, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is -G-P-P-V-S-C-I-K-R, the sequence thus being sequence No. 4 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

5. A peptide according to claim 4 which is cyclised through a disulphide bridge, the sequence thus being sequence No. 5 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

6. A peptide according to claim 1, wherein X_1 is none, X_2 is none, X_3 is A, X_4 is K, X_5 D, and $X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}$ is none, the sequence thus being sequence No. 6 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

7. A peptide according to claim 6, wherein a lactam bridge is formed between the amino acids K in position 5 and D in position 9, the sequence thus being sequence No. 7 given in the sequence listing, or functionally equivalent homologues or analogues thereof.

8. A medicinal product comprising a peptide according to any one of the claims 1-7.

9. A medicinal product according to claim 8 for treatment and/or prevention of infections, inflammations and/or tumours.

10. A medicinal product according to claim 9, for treatment and/or prevention of urinary tract infection.

11. A medicinal product according to claim 9, for treatment and/or prevention of colitis.

12. A medicinal product according to any one of the claims 8-11 formulated for oral administration.

13. A medicinal product according to any one of the claims 8-11 formulated for parenteral administration.

14. A medicinal product according to claim 13 formulated for topical administration.

15. A medicinal product according to any one of the claims 8-14 formulated for administration on mucosal membranes.

16. Food stuff comprising a peptide according to any one of claims 1-7.

17. Food stuff according to claim 16 being an infant formula food.

18. Use of a peptide according to any one of the claims 1-7 for the production of a medicinal product for treatment and/or prevention of infections, inflammations and/or tumours.

19. Use according to claims 18, wherein the medicinal product is intended for treatment and/or prevention of urinary tract infection.

20. Use according to claim 18, wherein the medicinal product is intended for treatment and/or prevention of colitis.

21. Use according to any one of the claims 18-20, wherein the medicinal product is formulated for oral administration.

22. Use according to any one of the claims 18-20, wherein the medicinal product is formulated for parenteral administration.

23. Use according to claim 22, wherein the medicinal product is formulated for topical administration.

24. Use according to any one of the claims 18-23 formulated for administration on mucosal membranes.

25. Use according to claim 21, wherein the medicinal product constitutes or is included a food stuff.

26. Use according to claim 25, wherein the food stuff is an infant formula food.

27. A method for treatment or prevention of infections, inflammations or tumours wherein an effective amount of a substance chosen from the group consisting of the peptides with sequences No. 1-7 given in the sequence listing and functionally equivalent homologues and analogues thereof is administered to a patient.

28. A method according to claim 27 for treatment and/or prevention of urinary tract infection.

29. A method according to claim 27 for treatment and/or prevention of colitis.

30. A method according to any one of the claims 27-29, wherein the substance is orally administered.

31. A method according to any one of the claims 27-29, wherein the substance is parenterally administered.

32. A method according to claim 31, wherein the substance is topically administered.

33. A method according to any one of the claims 27-32, wherein the substance is administered on mucosal membranes.

34. A method according to claim 30, wherein the substance is included in food stuff.

35. A method according to claim 34, wherein the substance is included in an infant formula food.

ABSTRACT

The invention relates to new linear and cyclic peptides the sequence of which is chosen from the group consisting of sequences No. 1-7 given in the sequence listing wherein X_1 is E or none, X_2 is A or none, X_3 is C or A, X_4 is Q or K, X_5 is N or D, and X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14} is -G-P-P-V-S-C-I-K-R or none, or functionally equivalent homologues or analogues thereof.

The invention also relates to medicinal products comprising such peptides, especially intended for treatment and prevention of infections, inflammations and tumours.

Furthermore, the invention relates to food stuff, especially infant formula food, comprising the above mentioned peptides.

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Huvudfaxen Kassan

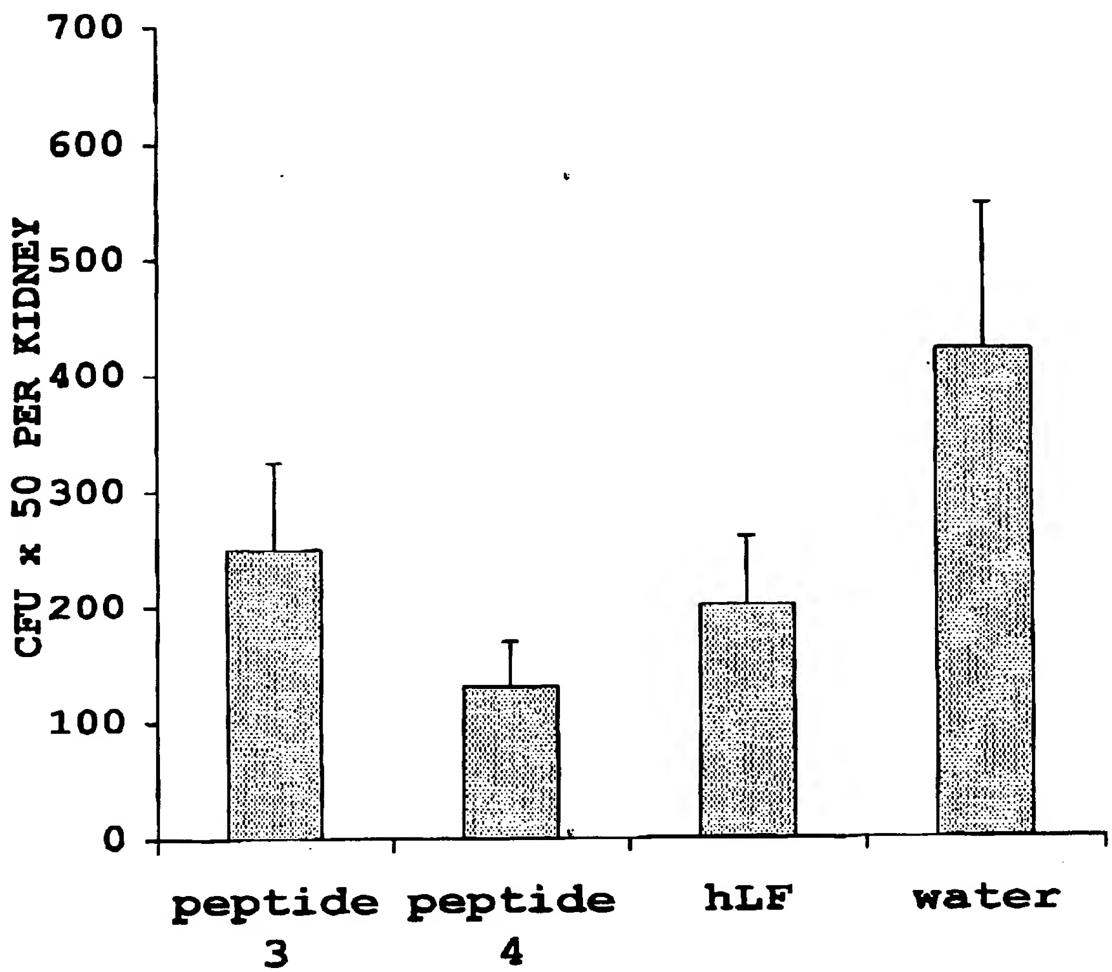

Figure 1